BROWNING OF SUGAR SOLUTIONS. IV. THE EFFECT OF pH ON THE VOLATILE PRODUCTS OF REDUCING SUGARS[a]

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The principal steam volatile carbonyl compounds obtained from the alkaline decomposition of reducing sugars are generally considered to be methylglyoxal and acetol. These compounds have also been suggested as browning intermediates *(22, 23)*. It is still doubtful, however, as to which of these two compounds represents the major product formed in the reaction. Fernbach and Schoen *(7)*, Fischler *(8, 9)*, Evans *(6)*, and Enders *(3, 4, 5)* have implicated methylglyoxal on the basis of the isolation of its phenylosazone. However, Sattler and Zerban *(16)* have questioned the use of this technique as positive proof of the presence of methylglyoxal since methylglyoxalosazone can also be formed from acetol. Baudische and Deuel *(1)* by means of a specific reaction of acetol with o-aminobenzaldehyde have indicated that it is acetol and not methylglyoxal which is produced in the distillation of glucose from weakly alkaline solution. Similarly, Nodzu *et al (12, 13)* based on the differential solubility of the semi-carbazone of acetol and methylglyoxal, also report the production of acetol but not methylglyoxal from several reducing sugars in mildly alkaline solutions. Prey and his co-workers *(14, 15)* using paper chromatography were able to demonstrate the formation of both acetol and methylglyoxal in alkaline solutions of glucose, dihydroxyacetone and glyceraldehyde. Similarly, Sattler and Zerban *(17)* have isolated acetol and a relatively lesser amount of methylglyoxal through solvent extraction of heated aqueous fructose solution.

During our investigation on color development in maple sirup, which is thought to occur through an alkaline degradation of the hexose constituents of the sap, several low molecular weight carbonyl compounds have been isolated as their dinitrophenyl-bis-hydrazones *(21)*. In an attempt to clarify further the nature of these compounds and their role in the mechanism of color and flavor development in the sirup, the authors have for some time been conducting experiments with model systems of dilute sugar solutions. In these preliminary studies it was observed that under varied experimental conditions, particularly of pH, the volatile constituents obtained from the sugars varied in both the nature of the compounds and in the amounts produced. This suggested that the pH of the reaction could explain why either acetol or methylglyoxal have been identified as the primary steam volatile products of the degradation. To clarify the extent to which these

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[b] From work done for a thesis submitted by H. G. Lento to the Graduate School of Georgetown University as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[c] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

compounds are produced, a study was made of the effect of pH on the formation of acetol and methylglyoxal from such reducing sugars as glucose, fructose, and dihydroxyacetone. This study required a means for distinguishing and estimating acetol and methylglyoxal when present in a mixture. Of the known methods of analysis for these compounds the procedures of Speck and co-workers *(10, 20)* were found to be the most specific and sensitive available. Methylgloxal is determined fluorometrically after its reactions with chromotropic acid and sulfuric acid. Acetol is also estimated fluorometrically after its reaction with o-aminobenzaldehyde in alkaline solution. Unfortunately, diacetyl interferes in both of these methods. Therefore, since this compound had been shown to be a volatile component in the distillates of reducing sugars *(13, 19)* it was necessary to eliminate diacetyl from solutions of acetol or methylglyoxal prior to their analysis. Recently Gabrielson and Samuelson *(11)* used an ion exchange column in the bisulfite form to remove and separate aldehydes and ketones in solutions of alcohol. Based on this, a method was developed using a bisulfite ion exchange technique for the quantitative separation of acetol, methylglyoxal and diacetyl when present in a mixture. This procedure has been applied to study the effect of pH on the production of these compounds from glucose, fructose and dihydroxyacetone.

EXPERIMENTAL

Materials. Acetol, methylglyoxal and diacetyl were obtained as "pure" compounds and further purified in the laboratory by vacuum distillation. Their concentration in the distillate was determined by periodic acid oxidation *(2)* and by precipitation with 2,4-dinitrophenylhydrazine.

Glucose, fructose and dihydroxyacetone were used without further purification as were all other chemicals and reagents used in these studies.

The phosphate buffer solutions were 0.25 molar disodium hydrogen phosphate adjusted to the desired pH with either one normal sodium hydroxide or with 85% phosphoric acid.

A Lumitron[a] photofluorometer equipped with a Corning No. 5874 red-purple primary filter and two secondary filters, Corning No. 3384 and Noviol 4308, was employed for the fluorescence measurements.

A Coleman Universal Colorimeter was used for colorimetric determination of diacetyl.

Chromatographic separation of acetol, diacetyl and methylglyoxal. Three grams of the anion exchanger IR400 (60-80 mesh) were washed with water to remove the suspended solids and packed as a wet slurry in a jacketed 15 mm ID glass column to a height of 25 cm. The column was fitted with a Teflon stopcock and a plug of glass wool to retain the resin. The temperature of the resin was kept at 4° C by circulating ice water through the jacket of the column so that the carbonyl compounds could be effectively retained as the bisulfite addition product.

The resin was placed in the hydroxyl form by treatment with 50 ml of 1.0 N NaOH. After washing the resin free of alkali with distilled water, 50 ml of a freshly prepared 0.2 N sodium bisulfite solution were added and the excess reagent removed with 100 ml of water. Twenty-five ml containing a known amount of acetol, methylglyoxal and diacetyl were added to the top of the ion exchange column and passed through it at the rate of 5 drops per minute. The complete retention of the carbonyls by the ion exchange resin was checked by testing the effluent with 2,4-dinitrophenylyhdrazine.

Five hundred ml of 0.05 N sodium chloride were used to elute the carbonyls from the resin. The eluate was collected in 10 ml fractions with an automatic fraction collector. A 1 ml aliquot of each of the 10 ml eluates was removed and tested with 2,4-

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[a] Mention of company and trade names does not imply endorsement by the Department over others not named.

dinitrophenylhydrazine to locate those fractions containing the carbonyls. Five ml of each of the consecutive tubes of the 3 groups giving a positive test were combined. A 1 ml aliquot was removed and tested qualitatively and quantitatively to identify and to determine the amount of compound present. The tests for methylglyoxal, diacetyl and acetol were those of Speck *(10, 18, 20)*. However, to test for acetol the bisulfite was first removed from the solution with sulfuric acid (0.03 ml). The test solution was freed from SO_2 by bubbling N_2 gas through it for several minutes. The solution was then neutralized with sodium hydroxide and the fluorometric determination of acetol performed. Table 1 shows the separation and recovery of acetol, methylglyoxal and diacetyl from mixtures obtained by the bisulfite ion exchange procedure.

TABLE 1

Separation of mixtures of carbonyls by bisulfite chromatography

Compound	Composition of mixture	Found	Recovery
	mg	*mg*	%
Acetol	2.00	1.92	96.0
Diacetyl	5.00	4.96	99.2
Methylglyoxal	0.80	0.82	101.1
Acetol	2.00	2.03	101.5
Diacetyl	0.20	0.19	95.0
Methylglyoxal	5.00	4.92	98.4

Distillation of glucose, fructose, and dihydroxyacetone. One hundred ml of an appropriate phosphate buffer solution of the desired pH was added to a 250 ml distillation flask in a heating mantle. The buffer was brought to boiling and an amount of either glucose (3.60 g), fructose (3.60 g), or dihydroxyacetone (1.80 g) to yield a 0.2 M solution was added. The buffered sugar solution was distilled and the first 2 ml were discarded. A 25 ml volumetric flask in an ice water batch was then used as the receiver and 25.0 ml of the distillate collected in approximately 8 min.

The 25 ml of distillate from each of the buffered sugar solutions was quantitatively transferred to the bisulfite ion exchange column and drained to the top of the resin. Five hundredths molar sodium chloride was then added to the column to elute the carbonyls. The effluent was collected in 10 ml portions and the fractions containing the acetol, diacetyl, and methylglyoxal were identified, combined and analyzed as previously described.

RESULTS AND DISCUSSION

By means of the bisulfite ion exchange column it was possible to separate from a mixture acetol, diacetyl, and methylglyoxal, as shown in Table 1.

Since acetol is a ketone which in general forms less stable bisulfite addition products, it is easily eluted from the column by sodium cloride. Diacetyl, a diketone, is more strongly bound to the bisulfite than is the acetol but less than the methylglyoxal which contains both an aldehyde and a ketone group. Thus, using sodium chloride as an eluting agent, acetol is the first to be removed followed by diacetyl and then by methylglyoxal. Table 1 also shows that mixtures of the 3 carbonyls can be separated quantitatively as well as qualitatively. Varying the relative amounts of diacetyl and methylglyoxal to acetol had little or no effect on the amount of recovery of these from their mixture. Further, these data demonstrate that the bisulfite ion exchange procedure is applicable to the analysis of sugar solutions (distillate) for acetol, diacetyl, and methylglyoxal.

Solutions of 3 sugars—glucose, fructose and dihydroxyacetone—each buffered at a different pH to cover the range of pH 4 to 11.8, were distilled and from the distillate acetol, diacetyl and methylglyoxal were separated and

determined. The concentration of these carbonyls in 25 ml of the distillate are plotted as the concentration versus pH of the sugar solutions to obtain the curves given in Figures 1, 2, and 3. The concentrations of the carbonyls are expressed logarithmically so that the large differences in amounts of the compounds produced can be shown.

Figure 1 shows that methylglyoxal is produced from solutions of dihydroxy-acetone buffered at pH 4 to 9 with the concentration of methylglyoxal increasing rapidly as the pH approaches 4. Likewise, Figure 1 shows that the amounts of both acetol and diacetyl produced from dihydroxyacetone buffered

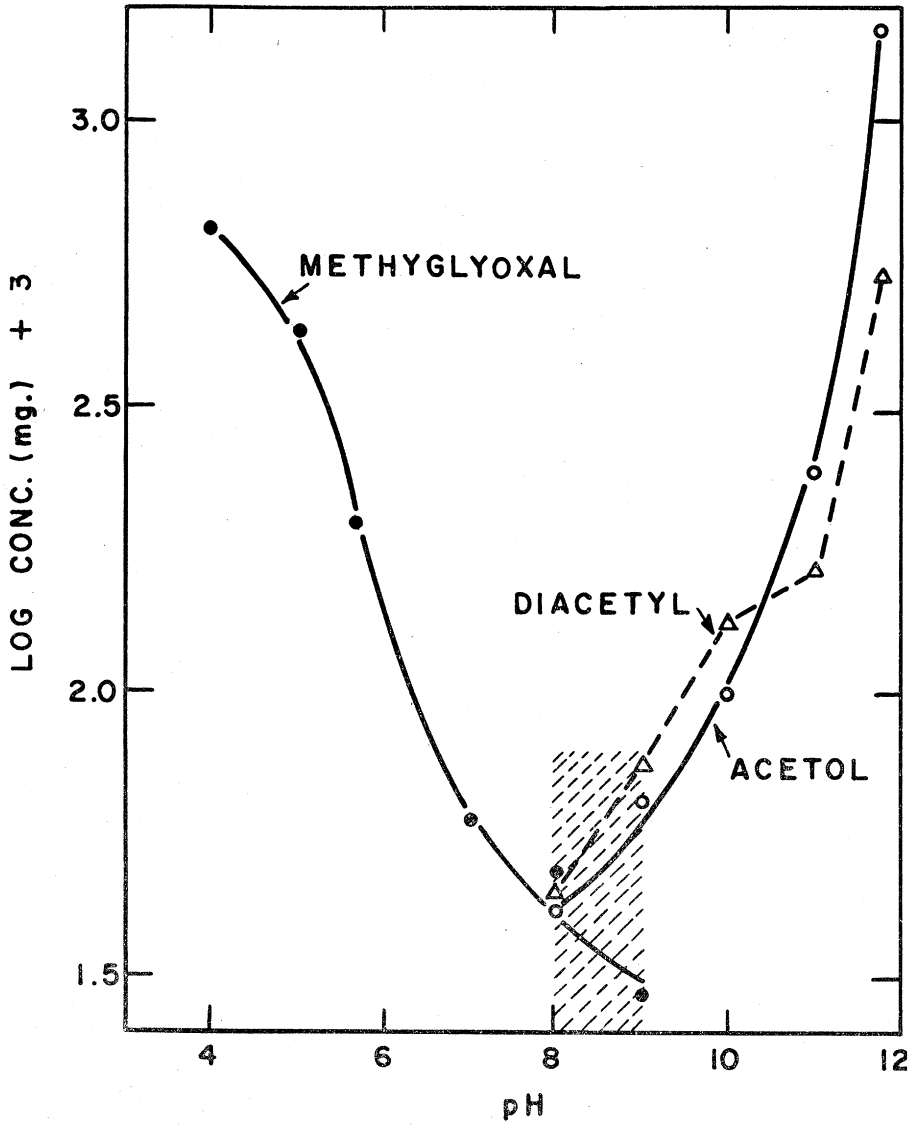

Figure 1. Effect of pH on the formation of methyglyoxal, diacetyl and acetol from dihydroxyacetone.

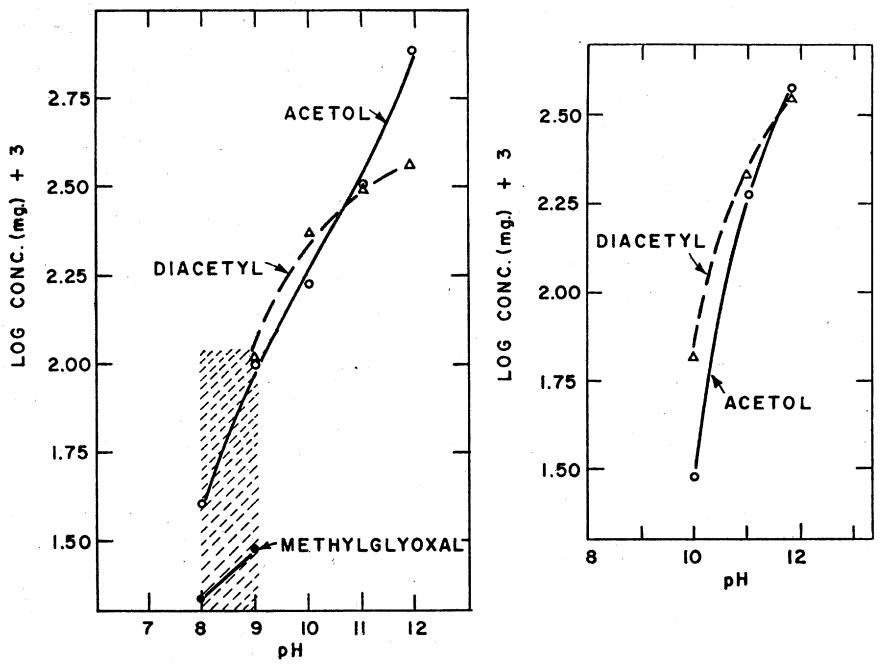

Figure 2. Effect of pH on the formation of methylglyoxal, diacetyl and acetol from fructose.

Figure 3. Effect of pH on the formation of methylglyoxal, diacetyl and acetol from glucose.

at pH 8 to 11.8 increases rapidly as the pH becomes more alkaline. The figure also shows that no detectable amounts (1.0 mg/liter) of acetol or diacetyl were found below pH 8, and above pH 9 no methylglyoxal was found.

Acetol and diacetyl are present in the distillates from alkaline solutions of fructose and glucose, as shown in Figures 2 and 3. Distillates from acid solutions of these 2 sugars yield no detectable amounts of methylglyoxal (0.8 mg/liter). Since it has been shown in Figure 1 that the distillates of acid solutions of dihydroxyacetone contain methylglyoxal, its absence from the distillates of glucose and fructose presents indirect evidence that heated acid solutions of either of these sugars do not favor the production of dihydroxyactone.

The shaded areas in Figures 1 and 2 indicate a pH range (8–9) in which all three of the carbonyls, acetol, diacetyl and methylglyoxal, are present in the distillates of either dihydroxyacetone or fructose. The lesser reactivity of glucose is demonstrated since these compounds were not detected in the distillate of this sugar in this pH range (Figure 3).

Although the amounts of acetol, diacetyl, and methylglyoxal (0.8 mg — 3 mg/liter) determined in the distillates from mild alkaline solution (pH 8–9) of fructose and dihydroxyacetone indicate that the production of these compounds is not favored, with more alkaline conditions relatively larger quantities of acetol and diaceyl are formed (Figures 1, 2), and with more acid conditions relatively more methylglyoxal is produced (Figure 1). Thus, the variation in both the distillate products and in the amounts of these products formed

emphasizes the effect which pH has on the production of acetol, diacetyl, and methylglyoxal from the reducing sugars. This effect is probably related to enolization, rearrangement, or fragmentation of the sugars caused by the pH of the solution.

CONCLUSIONS

By means of a bisulfite ion exchange procedure for the quantitative separation of acetol, methylglyoxal and diacetyl it has been possible to show that the factors which influence their formation are related to the particular reducing sugar involved and to the pH of the solution.

Increasing acidity favored the production of methylyglyoxal from dihydroxyacetone, with little or no acetol or diacetyl being formed from fructose, glucose, and dihydroxyacetone in acid solution. In strongly alkaline solutions (pH 10–12) acetol and diacetyl were produced from the three sugars. In mildly alkaline solutions (pH 8–9) relatively smaller amounts of acetol, diacetyl, and methylglyoxal were formed from both fructose and dihydroxyactone but not from glucose.

The production or non-production of the carbonyls, acetol, diacetyl, and methylglyoxal from these three sugars suggest that their formation is related to the ease with which heated solutions of sugars enolize, fragment or rearrange under different conditions of pH.

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